Cyclobutane Pyrimidine Dimers in UV-DNA Induce Release of Soluble Mediators that Activate the Human Immunodeficiency Virus Promoter

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Ultraviolet (UV) irradiation of human cells induced expression of a stably maintained fusion gene consisting of the human immunodeficiency virus long terminal repeat promoter controlling the bacterial chloramphenicol acetyltransferase gene. Two experiments demonstrated that DNA damage can initiate induction: UV induction was greater in DNA repair-deficient cells from a xeroderma pigmentosum patient than in repair-proficient cells, and transfection of UV-irradiated DNA into unirradiated cells activated gene expression. Increased repair of cyclobutane pyrimidine dimers by T4 endonuclease V abrogated viral gene activation, suggesting that dimers in DNA are one signal leading to increased gene expression. This signal was spread from UV-irradiated cells to unirradiated cells by co-cultivation, implicating the release of soluble factors. Irradiation of cells from DNA repair-deficiency diseases resulted in greater release of soluble factors than irradiation of cells from unaffected individuals. These results suggest that UV-induced cyclobutane pyrimidine dimers can activate the human immunodeficiency virus promoter at least in part by a signal-transduction pathway that includes secretion of soluble mediators. *J Invest Dermatol 100:790–794, 1993*

Localization of latent human immunodeficiency virus (HIV) in the skin is of concern because latent viruses can be activated by ultraviolet light (UV; reviewed in [1]). UV irradiation of cells carrying the HIV long terminal repeat (LTR) promoter linked to a reporter gene causes significant induction of HIV transcription, and UV irradiation of T-cell lines followed by HIV infection shortened the period until core antigens were detected [2]. In the murine-acquired immune deficiency syndrome model, UV exposure of mice at the time of infection by the LP-BM5 murine leukemia virus accelerated the disease symptoms including increased immunosuppression [3]. The induction has been related to DNA damage, because the HIV-LTR is activated by much lower doses of UV when it is resident in a cell from the DNA repair-deficient disease xeroderma pigmentosum (XP) than when it is in a repair-proficient cell [4].

The relevance of these studies to latent HIV infection has been explored in transgenic mice. Mice with the HIV-LTR linked to the *lac*Z gene were UV irradiated, and an increase in β-galactosidase was detected in skin [5]. Similarly, transgenic mice with the HIV-LTR controlling either the luciferase [6] or the *tat* [7] gene showed increased transcription after UV exposure. Most importantly, LTR induction was detected in both systems after the mice were exposed to sunlight for as little as 30 min.

UV activation of latent HIV-LTR transcription is clinically relevant to seropositive patients. Cutaneous lesions are frequently found on sunlight-exposed areas of the skin [8–10], and one study showed that seropositive patients had higher solar and artificial UV exposure than matched controls [11], having the mistaken belief that a suntan will improve their health. In addition, UV is a component of therapy for dermatologic diseases in AIDS such as eosinophilic folliculitis [12], and seropositive patients may receive iatrogenic UV exposure.

Our approach to studying UV activation of latent HIV-LTR is through the enhancement of DNA repair by delivery of exogenous DNA repair enzymes. T4N5 liposomes encapsulate T4 endonuclease V, a DNA repair enzyme that specifically initiates removal of UV-induced cyclobutane pyrimidine dimers (CPD) from DNA. T4N5 liposomes added to UV-irradiated human cells in culture stimulated the removal of CPD and increased DNA repair synthesis [13]. T4N5 liposomes in a lotion applied topically to hairless mice after UV exposure increased the removal of CPD from mouse epidermis and reduced the incidence of UV-induced skin cancer compared to untreated controls [14]. XP patients had faster removal of CPD from their skin treated with T4N5 liposomes than from untreated skin (J. Hawk, A. Young, and D. Yarosh, unpublished results).

MATERIALS AND METHODS

Cell Lines SV40-transformed human fibroblast strain GM637 and excision-repair-deficient strain XP12BE from a group A XP patient were supplied by the Coriell Institute for Medical Research (Camden, NJ). They were each transfected by the calcium phosphate method using plasmid pHIVcat-SVneo (a kind gift of Dr. Kristofer Valerie, Medical College of Virginia). GMHIV-2 and XPHIV-II were produced by selection with 800 μg/ml geneticin of transfected GM637 and XP12BE, respectively, and pooling resistant colonies. Fibroblast strain WI38 was obtained from Flow Laboratories (Costa Mesa, CA), and unaffected fibroblast line AG02804, Cockayne syndrome fibroblast line CRL-739 and basal cell nevus syndrome line CRL-1658 were from the Coriell Institute. Fibroblast lines were cultured as described [15] from skin biopsies: DD, NA, and RD were from basal cell carcinoma patients, and lines

Manuscript received November 2, 1992; accepted for publication February 3, 1993.

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Abbreviations: BCNS, basal cell nevus syndrome; CPD, cyclobutane pyrimidine dimers; CS, Cockayne's syndrome; LTR, long terminal repeat; XP, xeroderma pigmentosum.

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XP5FR and XP8FR were from two male XP patients of undetermined complementation groups. Transformed keratinocyte line A431 was from the American Type Culture Collection (Rockville, MD), and the T-cell leukemia line CEM was a kind gift of Dr. Tom Brent (St. Jude Children's Hospital, Memphis, TN).

Reagents Biotinylated rabbit anti-chloramphenicol acetyltransferase (CAT) and anti-neomycin phosphotransferase II (NeoPTII) antibodies were from 5′ → 3′ (Boulder, CO). Purified CAT was from Sigma (St. Louis, MO).

Liposomes T4N5 liposomes were prepared as described [16] and contained T4 endonuclease V encapsulated in membranes composed of phosphatidylcholine, phosphatidylethanolamine, oleic acid and cholesterol hemisuccinate (2:2:1:5 molar ratio). Liposomes for DNA transfection were prepared as described [17] using liposomes composed of phosphatidylethanolamine and dimethyldioctadecylammonium bromide. Plasmid pSV2gpt, in some cases after irradiation with 500 J/m² UV-C and treatment with 10 μg/ml T4 endonuclease V, was bound to the liposomes and immediately added to cells in media containing 4% serum. As a positive control, pRSVcat was used to measure the transfection efficiency.

UV Irradiation and Cell Culture Cells were cultured with Dulbecco's modified Eagle's medium prepared from powder (DME; GIBCO, Grand Island, NY) with 10% newborn calf serum (Irvine Scientific, Santa Ana, CA), 50 μg/ml gentamycin, and 0.5 μg/ml amphotericin B in 60-mm dishes to 80% confluence. They were irradiated without medium with UV-C from a Phillips G15T germicidal lamp emitting predominantly 254 nm light at a fluence rate of 1 J/m²/second, as monitored by a UVX digital radiometer (Ultraviolet Products, San Gabriel, CA). The cells were refed with media containing 10% serum and incubated for 18 h for direct determination of CAT activity. In experiments with liposomes the serum concentration in the media in both the experimental and control plates was reduced to 4%.

In transfer experiments, serum-free supernatants were collected from plates 48 h after UV irradiation, and the protein concentration determined by the Bradford reaction (Biorad, Richmond, CA). The supernatants were then mixed with serum-free DME, added to unirradiated XPHIV-II cells, and then incubated 48 h. In co-cultivation experiments, 10^5 untransfected cells were UV irradiated in 60-mm dishes and then 10^5 XPHIV-II cells in DME with 10% serum were added to the dish and co-incubated 48 h. In transwell experiments, 10^5 XPHIV-II cells were plated in 6-well 35-mm plates and 10^5 untransfected cells were plated onto 35-mm membrane inserts (Costar, Cambridge, MA) in separate plates and cultured overnight. The membrane-grown cells were UV irradiated and the inserts were then transferred to the wells containing the unirradiated XPHIV-II cells in 1 ml serum-free DME, and incubated 48 h. Liposomes were added to membrane-grown cells in serum-free media.

Chloramphenicol Acetyltransferase Assay Extracts were prepared from cells harvested in 0.25 M Tris, pH 7.8, by three cycles of freeze-thawing and protein concentration was determined by the Bradford reaction. CAT gene induction was measured using fluorescent BODIPY-labeled chloramphenicol (CAM) (Molecular Probes Inc., Eugene, OR) in an 80-μl reaction containing 5 nmol BODIPY-CAM; 0.25 M Tris, pH 7.8; 40 nmol acetyl CoA; and either 25 or 50 μg cell protein [18]. After 30 or 60 min the reaction products were extracted with ethyl acetate, spotted on silica thin layer chromatography plates and developed in 90% chloroform, 10% methanol. The images of the fluorescent plates, evenly illuminated with two black-light-blue 15-watt bulbs (predominantly 365 nm UV-A; General Electric, Cleveland, OH), were digitized by a Star I charge-coupled device digitizing camera (Photometrics Inc., Tucson, AZ) and stored as tagged-image-format computer files. The reaction products were quantitated from the digitized values for each fluorescent spot on the plate using QuantiScan version 1.1 software (Biosoft Ltd., Cambridge, UK). Each assay contained a positive control and acetylated BODIPY-CAM standards. CAT activity was calculated from the input nmol CAM and the fraction of acetylated products (corrected for background in the substrate), and is presented as the average nmol acetylated product per mg cell protein per hour for all measurements. Induced CAT activity is the activity in irradiated samples minus the activity in unirradiated samples.

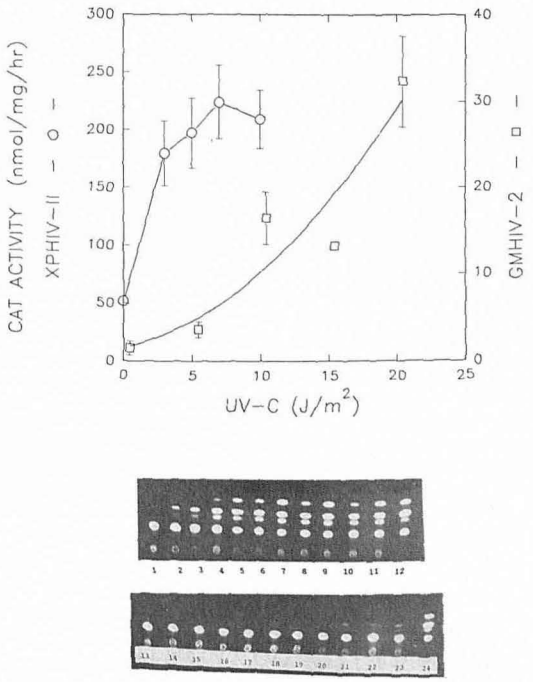

Figure 1. Induction of CAT activity as a function of UV-C irradiation. GMHIV-2 (□) and XPHIV-II (○) cells were irradiated with UV-C and then incubated for 18 h. *Top,* CAT activity is expressed as nmol acetylated CAM per mg cell protein per hour, and each *point* is the average of four measurements. *Error bars,* SEM. Bottom, representative thin-layer chromatogram of fluorescent BODIPY-CAT assay. *Lanes 1* and *13,* no extract; *lanes 2–11,* extracts of XPHIV-II treated in pairs of 25 and 50 μg protein with 0, 3, 5, 7, 10 J/m² UV-C; *lanes 14–23,* extracts of GMHIV-2 treated in pairs of 25 and 50 μg protein with 5, 10, 15, and 20 J/m² UV-C. *Lanes 12* and *24* contain standards of BODIPY-CAM and the acetylated forms.

Western Blotting Fifty micrograms of cell extracts prepared for CAT assay from the co-cultivation experiments were denatured by boiling with sodium dodecylsulfate (SDS) and electrophoresed through a 15% SDS-polyacrylamide gel with a 6% polyacrylamide stacking layer. The proteins were electrophoretically blotted to Immobilon P (Millipore, Bedford, MA) and probed with biotinylated rabbit antibodies to NeoPTII and CAT. Antibody binding was detected by avidin-alkaline phosphatase and 0.17 mg/ml nitro blue tetrazolium, 0.33 mg/ml 5-bromo-4-chloro-3-indoxyl phosphate in 0.1 M diethanolamine, pH 9.8.

RESULTS

Assay of UV Induction of HIV-CAT The CAT reporter gene system was used with fluorescent CAM to measure HIV LTR induction by UV [18]. Under our standard conditions, the production of acetylated CAM was linearly related to the input purified CAT enzyme activity up to 45% of the CAM acetylated by 62.5 units CAT (0.68% ± 0.02% CAM acetylated per unit CAT enzyme, correlation coefficient = 0.99).

The pHIVcatSVneo construct stably maintained in either the GMHIV-2 or the XPHIV-II fibroblasts was induced to express CAT activity by UV irradiation of the cells (Fig 1). The basal level of CAT expression in XPHIV-II cells was higher than that in GMHIV-2 cells (51.9 versus 1.5 nmol/mg/h), and UV-C increased activity in both cell lines. UV irradiation of XPHIV-II with up to 5

Table I. Induction of HIV-CAT in XPHIV-II Cell by Lipofection with UV-Irradiated DNA[a]

Treatment	Induced CAT Activity (nmol/mg/h)	Percent Induction	p Value
pSV2gpt	2.2 ± 2.4	21	>0.2
UV pSV2gpt	7.2 ± 3.4	70	0.05
UV-pSV2gpt/endo V	5.0 ± 5.6	48	>0.1
pRSVcat	10.4 ± 3.6	100	<0.01

[a] XPHIV-II cells were treated by lipofection with pSV2gpt DNA either unirradiated, irradiated with 500/m² UV-C, or irradiated and incubated with T4 endonuclease V. As a positive control cells were treated by lipofection with pRSVcat, which constitutively expresses CAT. After 18 h incubation the CAT activity was measured, the activity in the untreated control subtracted to determine the induced activity, and the result in nmol/mg/h is expressed with the standard error. The percent induction is calculated by comparison to pRSVcat, and the statistical significance of the difference from the untreated control (p value) was calculated by the Student two-tailed t test.

J/m² dramatically increased CAT activity, easily visible in the thin-layer chromatogram (Fig 1, *bottom, lanes 2–13*). At greater fluences the activity was in the non-linear response range of the standard reaction. In contrast, UV irradiation of repair proficient GMHIV-2 cells was much less dramatic at lower doses and clearly visible in the chromatograms only at 15 and 20 J/m² (Fig 1, *bottom, lanes 14–23*). Fluorescent image analysis of replicate experiments detected a steady increase in CAT activity in these cells up to 20 J/m² (Fig 1, *top*).

DNA Damage and Cyclobutane Pyrimidine Dimers The role of DNA damage was examined by introducing UV-C–irradiated plasmid pSV2gpt DNA into XPHIV-II by lipofection, and measuring HIV-LTR activation (Table I). As a positive control, cells were transfected with pRSVcat, which constitutively expressed CAT from the RSV promoter, and CAT induction from the HIV-LTR by UV-C was compared to expression in this control. XPHIV-II cells treated with unirradiated plasmid show a small increase in CAT activity that was not statistically significant. XPHIV-II cells treated with UV-irradiated plasmid had a statistically significant increase in CAT expression compared to untreated cell (70% of pRSVcat). This increase was abrogated by treating the UV-plasmid with T4 endonuclease V.

The importance of CPD was confirmed by treating UV-irradiated cells with T4N5 liposomes (Fig 2), which deliver T4 endonuclease V intracellularly and increase the removal of CPD, but not 6-4 pyrimidine-pyrimidone photoproducts, from DNA [13–16]. The liposomes produced an inhibition of UV-induced CAT gene expression in proportion to the liposome dose, and at the highest

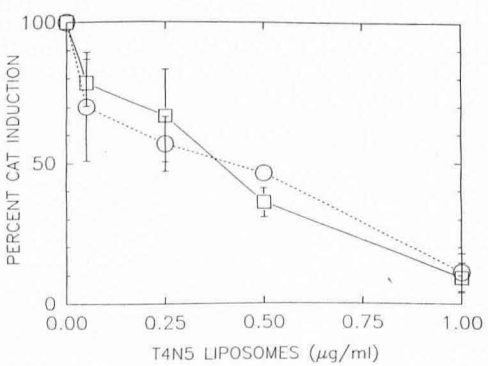

Figure 2. UV Induction of CAT activity as a function of T4N5 liposome concentration. GMHIV-2 (□) and XPHIV-II (○) cells were irradiated with 15 J/m² or 5 J/m² UV-C, respectively, and incubated for 18 h with T4N5 liposomes in media with 4% serum. CAT activity is expressed as a percent of activity in the absence of liposome treatment. *Error bars,* SEM.

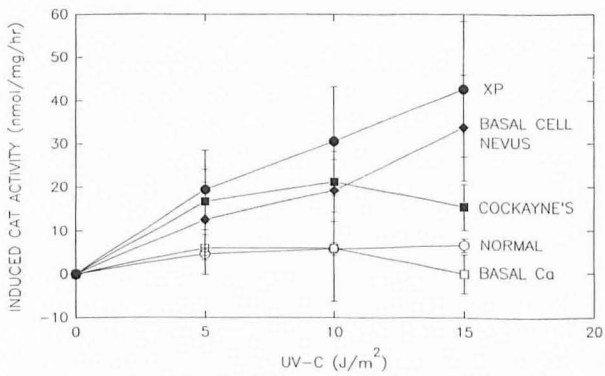

Figure 3. Induction of CAT activity in XPHIV-II cells as a function of UV irradiation to co-cultivated fibroblasts. Fibroblasts from four repair-proficient normal lines (○), four lines from patients with basal cell carcinoma (□), two lines from patients with XP (●), one line from a patient with Cockayne's syndrome (■), and one line from a patient with basal cell nevus syndrome (◆) were UV irradiated, and XPHIV-II cells were added and co-cultivated for 48 h, after which CAT activity was assayed. Induced CAT activity is that in UV-irradiated co-cultures minus that from unirradiated co-cultures. For each group of cell lines the induced CAT activity was averaged among the lines used. *Error bars,* SEM.

liposome doses the CAT activity after UV irradiation was only about 10% of that in the absence of T4N5 liposomes.

UV Induction of Soluble Mediators CPD are not required in the HIV-LTR DNA itself for CAT induction. Supernatant media from UV-irradiated XP fibroblasts transferred to unirradiated XPHIV-II cells produced CAT induction in proportion to the amount of protein in the conditioned media (data not shown).

A much stronger response was observed when UV-irradiated human skin fibroblasts were co-cultivated with unirradiated XPHIV-II cells (Fig 3). The greatest induction of CAT activity was observed when skin fibroblasts from either of two XP patients were irradiated with UV fluences of up to 15 J/m² and then added to dishes of XPHIV-II cells. This induction was greater than the induction by co-cultivation of UV-irradiated repair-proficient skin fibroblasts from either unaffected or nonmelanoma skin cancer patients with XPHIV-II cells ($p = 0.02$, by Mann-Whitney rank sum test). UV-irradiated fibroblasts from patients with other genetic photosensitivity diseases also produced greater CAT induction than unaffected fibroblasts upon co-incubation with unirradiated XPHIV-II cells. Fibroblasts from a Cockayne's syndrome (CS) patient and from a basal cell nevus syndrome (BCNS) patient each induced greater than normal CAT expression after UV (Fig 4). UV irradiation of the human keratinocyte line A431 and the human T-cell line CEM followed by co-cultivation with XPHIV-II cells also produced increased CAT activity (data not shown), indicating that the soluble factor(s) can be produced by several cell types.

We were concerned in these co-cultivation experiments that we might not be observing UV-induced release of an inducing signal, but rather UV killing of the irradiated fibroblasts and thus enrichment of XPHIV-II cells in the cell extract. To test for this artifact we examined the extracts from unirradiated and irradiated co-cultivated cells by Western blot for levels of the NeoPTII protein, constitutively produced by the *neo* geneticin-resistance gene in the XPHIV-II cells. These blots showed similar levels of the NeoPTII in unirradiated and irradiated cell extracts, demonstrating that the XPHIV-II cells were not selectively enriched in the irradiated extracts by killing of the co-cultivated fibroblasts (data not shown). In the same Western blots we found induction of the CAT protein after irradiation (data not shown).

The release of soluble mediators was further investigated in a transwell cell-culture system. XP fibroblasts were irradiated on membrane inserts with 0.2-micron pores, allowing passage of mole-

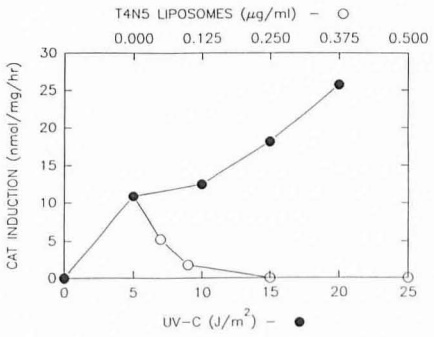

Figure 4. Induction of CAT activity in XPHIV-II cells by UV irradiation of XP12BE on membrane inserts, and inhibition by treatment with T4N5 liposomes. XP12BE cells on membrane inserts were irradiated with increasing doses of UV-C and incubated in transwells with XPHIV-II (●). The inserts were removed and CAT activity was measured in the XPHIV-II cells. XP12BE cells on membrane inserts were also irradiated with 5 J/m² UV-C, and treated with increasing concentrations of T4N5 liposomes (O) before incubation in transwells with XPHIV-II and CAT assay. The data represent the average of two experiments.

cules but not cells. The membranes were then inserted into cell-culture media above XPHIV-II cells attached to the bottom of the dish. UV irradiation of the XP fibroblasts on the membrane increased CAT activity in the unirradiated XPHIV-II cells in the dish (Fig 4). However, the induction was less than that observed in the co-cultivation experiments. Treatment of the UV-irradiated cells on the membrane with T4N5 liposomes reduced CAT activity in a liposome dose-dependent manner (Fig 4).

DISCUSSION

UV induces transcription from the HIV-LTR and the accumulated evidence demonstrates that DNA damage is able to activate this process. UV produced much greater gene expression when the HIV-CAT construct was resident in a DNA-repair deficient cell line than a normal line, and UV-irradiated plasmid DNA introduced into cells triggered CAT gene induction. However, this naked UV-DNA was an order of magnitude less efficient than direct irradiation of cells. Perhaps the structure of DNA (e.g., in chromatin), in addition to the amount of DNA damage, may be important in the induction process. The CPD produced by UV appears to be an important DNA lesion, because treatment of UV cells with the CPD-specific T4 endonuclease V liposomes or treatment of UV-DNA with pure T4 endonuclease V reduced CAT induction. The data suggest that an early step in UV-activation of HIV may include the formation of CPD in DNA.

The activating signal can be transferred from irradiated to non-irradiated cells. This was demonstrated using cell-free supernatants, co-cultivation of irradiated cells with unirradiated cells, and co-cultivation with separation by a microporous membrane. This implicates one or more soluble mediators that are very sensitive to dilution. The signal was strongest during co-cultivation in which UV- and unirradiated cells were in close physical proximity, lessened in the transwell experiments in which the signal passed from the membrane to the bottom of the dish, and weakest of all in cell-free supernatant transfer experiments, in which the signal was mixed evenly within the volume of the transferred media. The greatest signal was obtained by direct UV irradiation and this may indicate that an autocrine process is also involved. However, it may also suggest that CPD in the DNA containing the HIV-LTR increases transcription by cis-activation in addition to any soluble mediator acting by a trans-activation mechanism. It should be noted that UV induces CAT expression in XPHIV-II cells at UV fluences too low to produce CPD near the HIV-LTR sequences in more than a tiny fraction of cells.

The co-cultivation experiments provided an opportunity to examine the response of cells from patients with UV photosensitivity. Cells from XP patients produced much higher CAT activity after UV than normal cells, consistent with the defect in removal of CPD from DNA in XP. Cells from CS are thought to have a defect in the preferential removal of CPD from actively transcribed DNA [19] and they induced more CAT activity after UV than normals, but not as great as XP cells. Cells from BCNS patients are hypersensitive to UV-B [20], and may have a defect in repair of UV damage [21]. The disease predisposes to UV-induced skin cancers and UV irradiation of BCNS cells produced CAT activity comparable to CS. Cells from patients with nonmelanoma skin cancer but no genetic disease were indistinguishable from controls.

The three genetic diseases (XP, CS, and BCNS) often produce symptoms that are not obviously related to sun damage to epidermal DNA, such as neurologic and immunologic abnormalities (XP, CS) and bone and growth disorders (XP, CS, BCNS). The data presented here support our hypothesis that defective DNA repair may lead to abnormal cytokine release from UV-irradiated cells [22]. This in turn may lead to cutaneous photosensitivity and perhaps to noncutaneous symptoms at sites distant from the point of cytokine release.

The ability of UV to trigger HIV LTR transcription either by direct damage or by the release of soluble mediators has important implications in studying and managing HIV infection. The experiments reported here produced CPD using UV-C (which is filtered from sunlight by stratospheric ozone), but UV-B and sunlight also produce CPD. Although biologic effects of UV-C and UV-B differ, both UV-B and sunlight induce HIV expression in transgenic mice. Epidemiologic evidence relating solar UV exposure to shortened HIV latency is not available. However, UV and UV-A plus psoralen have been used in treatment of HIV-infected patients [18] and this may be a population to study for such a link. In the meantime, it would seem prudent to minimize the exposure of HIV-infected patients to solar or therapeutic UV.

We are grateful to Dr. Kristofer Valerie for the gift of pHIVcatSVneo and Dr. Tom Brent for the gift of the CEM cells. Special thanks to Dr. Margaret Kripke and Dr. Chris Hollander for discussions and critiques.

REFERENCES

1. Zmudzka B, Beer J: Activation of human immunodeficiency virus by ultraviolet radiation. Photochem Photobiol 52:1153–1162, 1990
2. Valerie K, Delers A, Bruck C, Thiriart C, Rosenberg H, Debouck C, Rosenberg M: Activation of human immunodeficiency virus type 1 by DNA damage in human cells. Nature 333:78–81, 1988
3. Brozek C, Shopp G, Ryan S, Gillespie P, Kusewitt D, Rajagopalan M, Ley K, Ley R: *In vivo* exposure to ultraviolet radiation enhances pathogenic effects of murine leukemia virus, LP-MB5, in murine acquired immunodeficiency syndrome. Photochem Photobiol 56:287–295, 1992
4. Stein B, Rahmsdorf H, Steffen A, Litfin M, Herrlich P: UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-*fos* and metallothionein. Mol Cell Biol 9:5169–5181, 1989
5. Cavard C, Zider A, Vernet M, Bennoun M, Saragosti S, Brimber C, Briand P: In vivo activation by ultraviolet rays of the human immunodeficiency virus type 1 long terminal repeat. J Clin Invest 86:1369–1374, 1990
6. Morrey J, Bourn S, Bunch T, Jackson M, Sidwells R, Barrows L, Daynos R, Rosen C: In vivo activation of human immunodeficiency virus type 1 long terminal repeat by UV type A (UV-A) plus psoralen and UV-B light in the skin of transgenic mice. J Virol 65:5045–5051, 1991
7. Vogel J, Cepeda M, Tschachler E, Napolitano L, Jay G: UV activation of human immunodeficiency virus gene expression in transgenic mice. J Virol 66:1–5, 1992

8. Mathes B, Douglass M: Seborrheic dermatitis in patients with acquired immunodeficiency syndrome. J Am Acad Dermatol 13:947–951, 1985
9. Safai B, Johnson K, Myskowski P, Koziner S, Yang S, Cunningham-Rundles S, Godbold J, Dupont B: The natural history of Kaposi's sarcoma in the acquired immunodeficiency syndrome. Ann Intern Med 103:744–750, 1985
10. Sadick N, McNutt N, Kaplan M: Papulosquamous dermatosis of AIDS. J Am Acad Dermatol 22:1270–1277, 1990
11. Flegg P: Potential risks of ultraviolet radiation in HIV infection. Int J Study Aids 1:46–48, 1990
12. Buchness M, Lim H, Hatcher V, Sanchez M, Soter N: Eosinophilic pustular folliculitis in the acquired immunodeficiency syndrome. N Engl J Med 318:1183–1186, 1988
13. Kibitel J, Yee V, Yarosh D: Enhancement of ultraviolet-DNA repair in *den*V gene transfectants and T4 endonuclease V-liposome recipients. Photochem Photobiol 54:753–760, 1991
14. Yarosh D, Alas L, Yee V, Oberyszyn A, Kibitel J, Mitchell D, Rosenstein R, Spinowitz A, Citron M: Pyrimidine dimer removal enhanced by DNA repair liposomes reduces the incidence of UV skin cancer in mice. Cancer Res 52:4227–4231, 1992
15. Yarosh D, Kibitel J, Green L, Spinowitz A: Enhanced unscheduled DNA synthesis in UV-irradiated human skin explants treated with T4N5 liposomes. J Invest Dermatol 97:147–150, 1991
16. Yarosh D, Tsimis J, Yee V: Enhancement of DNA repair of UV damage in mouse and human skin by liposomes containing a DNA repair enzyme. J Soc Cosmet Chem 41:85–92, 1990
17. Rose J, Buonocore L, Whitt M: A new cationic liposome reagent mediating nearly quantitative transfection of animal cells. BioTechniques 10:520–525, 1991
18. Young S, Barbera L, Kaynard A, Haugland R, Kang H, Brinkley M, Melner M: A nonradioactive assay for transfected chloramphenicol acetyltransferase activity using fluorescent substrates. Anal Biochem 197:401–407, 1991
19. Venema J, Mullenders L, Natarajan A, van Zeeland A, Mayne L: The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc Natl Acad Sci USA 87:4707–4711, 1990
20. Applegate L, Goldberg L, Ley R, Ananthaswamy II: Hypersensitivity of skin fibroblasts from basal cell nevus syndrome patients to killing by ultraviolet B but not by ultraviolet C radiation. Cancer Res 50:637–641, 1990
21. Nagasawa H, Burke M, Little F, McClone E, Chan G, Little J: Multiple abnormalities in the ultraviolet light response of cultured fibroblasts derived from patients with the basal cell nevus syndrome. Terat Carcinog Mutagen 8:25–33, 1988
22. Yarosh D: The role of DNA damage and UV-induced cytokines in skin cancer. J Photochem Photobiol B:Biol 16:91–94, 1992